Available online at www.sciencedirect.com
SCIENCE DIRECT®
Mutation Research 558 (2004) 155–167
Genetic Toxicology and Environmental Mutagenesis
www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Genotoxic and mutagenic activity of environmental air samples in Flanders, Belgium

V.A. Du Four [a,*], N. Van Larebeke [b], C.R. Janssen [a]

[a] *Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Ghent, Belgium*
[b] *Department of Radiotherapy, Nuclear medicine and Experimental Cancerology, Study Center for Carcinogenesis and Primary Prevention of Cancer, Ghent University, Ghent, Belgium*

Received 26 August 2003; received in revised form 24 November 2003; accepted 1 December 2003

Abstract

Atmospheric pollution is assumed to play a role in the incidence of respiratory diseases and cancers. Airborne particles are able to penetrate deep into the lung and are composed of complex chemical mixtures, including mutagens and carcinogens such as polycyclic aromatic compounds (PACs). The present study reports mutagenic and genotoxic activities associated with ambient air collected near a busy street in Borgerhout, at an industrial site in Hoboken and in Peer, a rural community 70 km east of Antwerp in Flanders, Belgium. Airborne particulates (PM10) and semi-volatile organic compounds were sampled during winter and summer. Samples were collected with a high-volume sampler using quartz filters (QF) and polyurethane foam (PUF) cartridges. The mutagenic and genotoxic activity of the organic extracts was determined using the *Salmonella* test/standard plate-incorporation assay and the Vitotox® assay. Concentrations of 16 polycyclic aromatic hydrocarbons (PAHs) in the extracts were determined by reversed-phase high-performance liquid chromatography (HPLC). The mutagenicity assay, using *Salmonella typhimurium* strain TA98, demonstrated direct mutagenicity of up to 58 revertants/m^3 for the QF extracts and low or no mutagenic activity in the PUF extracts. Metabolic activation of the samples resulted in high indirect mutagenicity for both QF and PUF extracts: up to 96 revertants/m^3 were found in QF samples and 62 revertants/m^3 in PUF samples. Genotoxic effects of the filter extracts were assessed with the Vitotox® assay: some direct genotoxic effects were noted, i.e. without metabolic activation, but almost no effects were observed after metabolic activation. Without activation, most PUF extracts were bacteriotoxic. With metabolic activation this toxicity disappeared, but genotoxic effects were not observed. Statistical analysis showed that the observed biological effects correlated well with the PAH concentrations.

Keywords: Airborne particles (PM10); *Salmonella* mutagenicity assay; Genotoxicity; Polycyclic aromatic hydrocarbons; Vitotox® assay

1. Introduction

Residents of industrialised and densely populated regions, like Flanders (Belgium), are exposed to ambient air pollution arising primarily from industrial activities, traffic sources and heating. Epidemiological studies, carried out to investigate the health risks related to air pollution, suggest that ambient air pollution may be responsible for increased rates of lung cancer [1–3]. Particulate matter with a mean diameter less than 10 μm (PM10) is associated with adverse health effects including increased respiratory problems, cancer and mortality [4]. Indeed, PM10 particles have the ability to penetrate and deposit

* Corresponding author. Tel.: +32-9-264-37-10; fax: +32-9-267-37-66.
E-mail address: vincent.dufour@ugent.be (V.A. Du Four).

1383-5718/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.mrgentox.2003.12.002

in the tracheo-bronchial and alveolar regions of the respiratory tract [5]. Various air pollutants/organic compounds that are present in the air in a gaseous and semi-volatile state are known to adsorb onto the surface of these airborne particles. Of primary concern are the PAHs and their derivatives, such as nitro-PAHs (NPAHs) and oxy-PAHs, collectively known as polycyclic aromatic compounds (PACs). Several PAHs and NPAHs are considered as probable (2A) or possible (2B) human carcinogens by the International Agency for Research on Cancer (IARC) [6].

Identifying and estimating human exposure to these mutagenic and genotoxic compounds is imperative in evaluating public health risk. However, the complexity and the potentially interactive effects of airborne toxic compounds cannot be adequately ascertained by chemical analysis. Therefore, bio-monitoring of environmental air to assess its mutagenic activity in addition to conventional chemical monitoring is receiving increasing attention for evaluating potential risks to public health [7,8].

Little or no information is available on the levels of airborne mutagenicity/genotoxicity in Flanders. In the present study the mutagenic and genotoxic activities associated with ambient air were evaluated using two bioassays: the *Salmonella* test/plate-incorporation assay and the Vitotox® test. Airborne particles (PM10) and semi-volatile organic compounds were collected during winter and summer near a busy street in Borgerhout, at an industrial site in Hoboken and in Peer, a rural community 70 km east of Antwerp. These samples were analysed for PAH and bioassays were performed to determine their genotoxic and mutagenic activity. In addition, the suitability of the bioassays for monitoring purposes was evaluated. Finally, PAH concentrations and mutagenic/genotoxic activities were statistically compared to investigate potential relations between observed biological effects and PAH concentrations.

2. Experimental: materials and methods

2.1. Sampling and sample preparation

The air samples were collected during winter 2000–2001 and summer 2001. Three different sites, representative of different sources of emission, were selected. The urban site in 'Borgerhout' was located close to a busy approach road to the centre of Antwerp, with intense traffic including many diesel vehicles. The industrial site 'Hoboken', a suburb of Antwerp, was located next to a large non-ferrous smelter; Hoboken also harbours metallurgic, printer and electronic equipment industries. 'Peer', situated in a rural area 70 km east of Antwerp, was selected to represent background conditions in Flanders with low inputs from industry and heavy traffic (no polluting industries within 15 km, no highway). Each of these sites (urban, industrial and rural) was sampled three times in the winter and three times in the summer, resulting in 36 samples (18 QF and 18 PUF, see below).

The samples were collected with a high-volume air sampler (Digitel) with a size-selective inlet (PM10). The sampler inlet was located at street level (1.8 m height). Particulate matter and semi-volatile compounds were collected on quartz filters (QF20 Schleicher & Schuell) and polyurethane foam (PUF) cartridges, placed in series. The more volatile compounds, which were not trapped on the filter, were retained in the PUF cartridges. These had been pre-cleaned by 24 h Soxhlet extractions using acetone (CAS 67-61-1). The samples were taken at a flow rate of 30 m^3/h (500 l/min) during 48 h, resulting in a total sampled air volume of about 1300 m^3. All samples were taken during a weekday starting at about 9 a.m. The quartz filters were changed automatically after 24 h. The same PUF cartridge, however, was used during the entire 48 h sampling. The weight of the collected particulate matter on the filters was determined by weighing the filters before and after sampling (prior to weighing, 24 h conditioning at 20 °C in a desiccator). Sampled filters were stored at −18 °C until extraction.

QF and PUF were extracted separately in a Soxhlet apparatus during 24 h with 100 and 2000 ml acetone (CAS 67-61-1). QF and PUF blanks were extracted in an identical manner. Individual extracts were concentrated to 5.0 ml under a gentle stream of N_2 with a turbovap concentrator. A 0.5 ml aliquot was taken for high-pressure liquid chromatography (HPLC) analyses, the remaining volume was evaporated and the residue dissolved in 4.5 ml dimethyl sulfoxide (DMSO) (CAS 67-68-5) and evaluated in the bioassays. The extracts were stored at −80 °C prior to the bioassay.

2.2. PAH analyses

The organic extracts of the QF and PUF cartridges were analysed for the presence of 16 PAHs using HPLC in combination with fluorescence detection [9]. The detected and quantified PAHs (three to five rings) were: naphthalene (N), acenaphthylene (ACY), acenaphtene (ACE), fluorene (FL), phenanthrene (PH), anthracene (AN), fluoranthane (F), pyrene (P), benzo(a)anthracene (B[a]A), chrysene (CH), benzo(b) fluoranthane (B[b]F), benzo(k)fluoranthane (B[k]F), benzo(a)pyrene (B[a]P), dibenzo(a,h)anthracene (DB[a,h]A), benzo(g,h,i)perylene (B[g,h,i]P) and indeno(1,2,3-c,d)pyrene (I[c,d]P).

A 0.5 ml aliquot of each organic extract was solvent-exchanged into 1 ml acetonitrile (CAS 75-05-8). The HPLC system consisted of a liquid chromatography system (Waters, Milford, MA, USA) and a fluorescence detector (Perkin-Elmer LC240). Separation of the PAHs was accomplished using a Vydac 201TP column (250 mm × 4.6 mm), with gradient elution ranging from a 50/50 acetonitrile–water mixture to 100% acetonitrile (CAS 45-05-8) in 20 min. The fluorescence of PAHs was monitored with automatic adjustment of the wavelength for each compound according to the retention time. The excitation wavelengths ranged between 260 and 300 nm, the emission wavelengths between 380 and 465 nm. The standard reference material SRM1647 (NIST) was used for calibration of the quantification method.

2.3. Bioassays

The organic extracts of QF and PUF cartridges, dissolved in DMSO, were tested for their mutagenic and genotoxic activity using two different bioassays.

The *Salmonella* test is a well-known bacterial mutagenicity assay where the reverse $His^- \rightarrow His^+$ mutations are visualised by plating *Salmonella typhimurium* bacteria in a histidine-poor growth medium [10]. The assays were performed on both the QF and PUF extracts using the standard plate-incorporation test [11,12] with *S. typhimurium* strain TA 98, in the presence and absence of an exogenous metabolic activation system (S9). The effects measured following metabolic activation are considered as the indirect mutagenicity. The effects measured without activation are regarded as direct mutagenicity. Dimethyl sulphoxide (DMSO) was used as a solvent control, whereas 4-nitroquinoline-N-oxide (0.7 μg per plate) (CAS 56-57-5) and benzo(a)pyrene (5 μg per plate) (CAS 50-32-8) were used as positive controls without (−S9) and with (+S9) exogenous metabolic activation, respectively. For the exogenous metabolic activation a 10% S9 mix was prepared with Aroclor 1254-induced rat liver S9 homogenate (ICN) [12]. Each sample was assayed in three concentrations (due to limited test material) using three replica plates per concentration. Revertants were counted using Quantity One quantification software in combination with Gel Doc2000 equipment (Bio-Rad), after 48 h incubation at 37 °C. The data obtained are presented as revertants/m^3 of air sampled, calculated from the dose–response curves. Samples giving at least a two-fold increase of mutants (for the highest test concentration) compared with the average yield of spontaneous revertants (TA98: number of spontaneous revertants between 20 and 50) and with a concentration-related response, were regarded as positive [12].

The Vitotox® test is a bacterial assay, similar to the SOS chromotest [13], based on the expression of repair genes induced by genotoxic agents. This bacterial SOS system is a DNA repair mechanism that reacts directly and immediately to DNA damage. The bacterial strain used for this assay is TA104 RecN2-4 [14], which has a *lux* operon of the luminescent marine microorganism *Vibrio fischeri* under transcriptional control of the recN gene, which is a part of the SOS repair system. Under the influence of a genotoxic compound, the RecN promoter is depressed, which results in expression of the *lux* operon and light production. Some chemicals directly interfere with the light-emission system and stimulate light emission without any genotoxic activity (false positives). Other chemicals are bacteriotoxic and decrease light intensity because the microorganisms are killed. In this case genotoxic effects might be masked (false negatives). To estimate these effects, the test substance is simultaneously tested on a constitutive bacterial strain, TA104 pr1 [15]. This construct provides the organisms with a background light emission. Substances that interfere directly with light emission will be detected with this strain and the genotoxic response can be corrected for this direct interference.

The Vitotox® test is performed in 96-multiwell plates filled with the bacterial mix and dilutions of the test substance. Dilution series are prepared in the

presence and absence of a microsomal enzymatic mixture S9 (see above). Luminescence is measured with an automated spectrophotometer. Results are analysed with a standard software package (Microsoft-Excel), which calculates for each concentration:

(1) the maximal signal-to-noise ratio for the Rec strain (i.e. maximal luminescence of the treated cells, divided by the maximal luminescence of the untreated cells);
(2) the maximal signal-to-noise ratio for the pr1 strain (i.e. maximal luminescence of the treated cells, divided by the maximal luminescence of the untreated cells);
(3) the ratio of (1) and (2), which is used to correct for toxicity and for direct induction of the *lux* genes (max S/N(recN2-4)/max S/N(pr1)).

Genotoxicity is assumed to be present when the results of calculations (1) and (3) are equal to or larger than 1.5, and when there is a good concentration–effect relationship [15].

2.4. Statistics and correlation analyses

For data analyses of the environmental samples, non-parametric tests were selected because these do not require a normal distribution and a homogeneous variance. The non-parametric Wilcoxon matched pairs test was used for testing paired data, correlations were tested using the non-parametric Spearman test (Statistica, Statsoft, 2000) [16]. The mutagenic and genotoxic responses were related to the analyses of the 16 PAHs using partial least-squares projections to latent structures (PLS) models Simca-P 9.0 software (Umetrics, Umeå, Sweden) [17]. PLS models allow an investigation of correlations between numerous, often correlated input and process variables (X) and several result variables (Y). PLS analysis results in model coefficients for the variables, called weights. The weights for the X variables indicate the importance of these variables in the modelling of Y. The R^2 of the model is a measure for the variance explained by the model, while Q^2 is a measure for the variance of the variables that can be predicted by the model. The resulting model can be used to predict the mutagenic or genotoxic responses based on measured chemical concentrations [18,19]. A first model was developed with all 16 input variables (PAHs). Next, the known indirect-acting mutagens, i.e. 10 of 16 analysed PAHs, were related with the indirect mutagenic activity (+S9). Resulting models were evaluated with respect to their correlation coefficients (R^2) and prediction properties (Q^2).

3. Results

3.1. Particulate matter

The amounts of airborne particles (PM10) collected on the filters are given in Table 1. The amount ranged from 12 to 57 $\mu g/m^3$ with an average of $32.8 \pm 3.6\ \mu g/m^3$. The average PM10 concentrations were not significantly different among the three sites. There was no significant difference between the PM10 concentrations in winter and summer. Observed difference in the collected amounts are due to differences in the meteorological conditions (i.e. rain, wind).

3.2. Chemical analyses

The concentrations of the 16 PAHs in the organic extracts (QF + PUF) are shown in Table 2. PAH

Table 1
Amount of particle matter (PM10) collected on filters

Location	Season[a]	Total (mg)	Concentration ($\mu g/m^3$)
Urban	W 1	48.0	25.4
	W 2	57.8	45.3
	W 3	42.1	32.9
	S 1	52.4	41.6
	S 2	45.8	36.1
	S 3	39.5	31.4
Industrial	W 1	58.5	45.7
	W 2	44.5	34.2
	W 3	34.7	27.1
	S 1	48.3	38.3
	S 2	44.2	34.9
	S 3	15.9	12.1
Rural	W 1	39.6	30.3
	W 2	75	56.9
	W 3	18.3	14.3
	S 1	62.0	49.4
	S 2	24.7	19.4
	S 3	20.0	15.7

[a] Each location was sampled three times in winter (W) and summer (S).

Table 2
Concentrations (mean ± S.D.) of PAHs in the organic extracts (QF + PUF)[a]

PAHs	Mean[b] (ng/m^3) ± S.D.					
	Urban		Industrial		Rural	
	Winter	Summer	Winter	Summer	Winter	Summer
Naphthalene	2.96 ± 2.94	4.32 ± 1.51	0.55 ± 0.31	1.66 ± 0.94	6.52 ± 10.53	1.34 ± 1.02
Acenaphthylene	5.64 ± 4.88	0.58 ± 0.48	2.88 ± 3.09	0.23 ± 0.20	23.7 ± 31.5	0.17 ± 0.16
Acenaphtene	0.39 ± 0.6	0.59 ± 0.17	0.04 ± 0.03	0.39 ± 0.33	0.15 ± 0.09	1.17 ± 1.67
Fluorene	11.5 ± 3.41	6.36 ± 2.01	8.78 ± 5.17	3.57 ± 2.05	16.1 ± 11.64	3.33 ± 1.71
Phenanthrene	49.4 ± 16.05	51.9 ± 6.68	27.8 ± 8.95	31.3 ± 21.25	44.8 ± 19.03	21.1 ± 9.41
Anthracene	6.22 ± 2.32	1.31 ± 0.08	2.5 ± 1.67	0.4 ± 0.19	6.05 ± 3.22	0.29 ± 0.1
Fluoranthane	9.76 ± 2.45	5.85 ± 3.11	7.56 ± 2.81	2.81 ± 1.43	17.9 ± 8.8	3.38 ± 1.89
Pyrene	13 ± 4.38	3.11 ± 0.66	6.8 ± 2.06	1.64 ± 0.16	11.8 ± 5.58	1.4 ± 0.64
Benz(a)anthracene	0.41 ± 0.05	0.19 ± 0.14	0.37 ± 0.54	0.59 ± 0.9	1.76 ± 1.57	0.13 ± 0.14
Chrysene	1.84 ± 0.5	0.41 ± 0.2	1.6 ± 1.65	0.53 ± 0.46	5.39 ± 4.38	0.15 ± 0.10
Benzo(b)fluoranthane	1.46 ± 0.45	0.3 ± 0.14	1.58 ± 1.61	0.15 ± 0.08	4.04 ± 2.61	0.21 ± 0.18
Benzo(k)fluoranthane	0.84 ± 0.29	0.15 ± 0.07	0.8 ± 0.65	0.05 ± 0.04	1.9 ± 1.29	0.07 ± 0.04
Benzo(a)pyrene	0.82 ± 0.31	0.07 ± 0.01	0.76 ± 1.04	0.03 ± 0.02	2.53 ± 2.41	0.03 ± 0.02
Dibenzo(a,h)anthracene	0.43 ± 0.18	0.42 ± 0.26	0.6 ± 0.7	0.54 ± 0.47	0.88 ± 0.38	0.53 ± 0.79
Benzo(g,h,i)perylene	2.18 ± 0.77	1.3 ± 0.26	1.82 ± 1.34	0.72 ± 0.5	3.63 ± 2.64	0.48 ± 0.56
Indeno(1,2,3-c,d)pyrene	1.62 ± 0.54	0.72 ± 0.24	1.76 ± 1.76	0.42 ± 0.25	4.41 ± 3.3	0.33 ± 0.34

[a] Sampler types—QF: quartz filter; PUF: polyurethane foam.
[b] Values are the mean ± S.D. of three samples.

concentrations in the winter were significantly higher (except for ACE) than in the summer ($P = 0.0006$). Analysis of the PAH profiles showed that concentrations of the low molecular weight (up to pyrene, mw 202.26) were 5–10 times higher than the high molecular weight (from B[a]A, mw 228) PAHs. FL, PH, F and P were the most abundant low molecular weight PAHs, and CH, B[b]F and I[c,d]P were most abundant of the high molecular weight PAHs. The PAH concentrations varied between the different locations. For the winter samples, the mean ($n = 3$) total concentration ($\sum$16 PAHs) ranged from 66.5 ± 31.3 ng/m^3 in the industrial site to 108.5 ± 34.7 ng/m^3 in the urban location and 154.2 ± 101.8 ng/m^3 in the rural location. During the summer, these concentrations ranged from 45.1 ± 25.8 to 77.6 ± 10.7 and 34.1 ± 18.2 ng/m^3, respectively.

The concentrations of the 16 PAHs found in the filter extracts differed from those in the PUF extracts. The partitioning of the individual PAHs over the QF and PUF are shown in Fig. 1. For the winter samples, the two- to four-ring PAHs (from N to P) were mainly present in the PUF extract, whereas the heavier four- and five-ring PAHs (starting with B[a]A) were mainly retained in the filter extracts. In the summer, the low molecular weight PAHs were almost exclusively found in the PUF extracts, whereas the high molecular weight PAHs were found both in the QF and in the PUF extracts. In the summer, concentrations of DB[a,h]A, B[g,h,i]P and I[c,d]P were higher in the PUF extracts than in the QF extracts.

3.3. Mutagenic activity assessed by the Salmonella test

Results of the mutagenicity assay of the QF and PUF extracts are shown in Table 3. Results are expressed as specific mutagenicity, i.e. revertants per metrecube (±95% confidence limits) obtained from the dose–response curves by regression analyses (Statistica, Statsoft, 2000) and revertants per microgram PM10 (QF samples only). Due to limited amounts of test material, only three test doses were used, therefore the resulting regression analyses (slope) could be influenced. The raw data (mean number of revertants per dose ± S.D.) is available on the web site: http://allserv.ugent.be/~vdufour.

The direct (−S9) and the indirect (+S9) mutagenicity differed with sample type (QF or PUF) and season. Most filter extracts (except industrial summer 3 and rural winter 3, without activation) showed

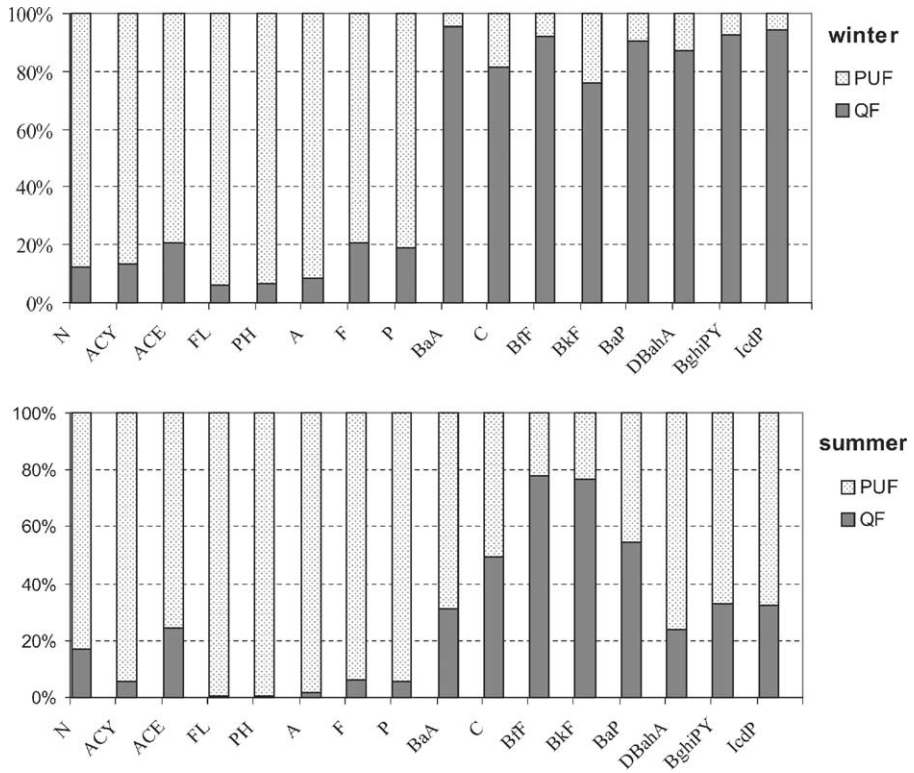

Fig. 1. Relative concentrations of the 16 analysed PAHs on the quartz filter and polyurethane foam cartridge in winter and summer samples. PAHs—N: naphthalene, ACY: acenaphthylene, ACE: acenaphtene, FL: fluorene, PH: phenanthrene, AN: anthracene, F: fluoranthane, P: pyrene, B[a]A: benzo(a)anthracene, CH: chrysene, B[b]F: benzo(b)fluoranthane, B[k]F: benzo(k)fluoranthane, B[a]P: benzo(a)pyrene, DB[a,h]A: dibenzo(a,h)anthracene, B[g,h,i]P: benzo(g,h,i)perylene and I[c,d]P: indeno(1,2,3-c,d)pyrene.

mutagenic activity with and without metabolic activation. All PUF extracts exhibited indirect mutagenicity and a few also showed direct mutagenicity. In winter samples, the indirect activity of the material from the filters was significantly higher than the direct activity ($P = 0.018$), whereas no significant difference was observed in samples collected during the summer. Addition of S9 to the PUF extracts resulted in higher activities, both for samples taken during winter and summer ($P = 0.002$).

The mutagenic activity in winter and summer depended on the sample type: the QF samples had the highest activity in the winter whereas the PUF samples had the highest activity in the summer ($P = 0.028$). However, the total (QF + PUF) direct and indirect mutagenicity was significantly higher ($P = 0.028$) in winter than in summer. The mean total activity for the three sites, considering both direct and indirect mutagenicity, was 45.8 ± 24.4 revertants/m^3 during the winter and 29.1 ± 19.2 revertants/m^3 in the summer.

The average mutagenic activity of the ambient air at the three locations was very similar; only with respect to direct mutagenic activity in the summer period a higher activity of 39.6 ± 3.7 revertants/m^3 was measured in Borgerhout (urban) compared to 18.8 ± 14 revertants/m^3 in Hoboken (industry) or 19.6 ± 19.8 revertants/m^3 in Peer (rural).

The highest total responses were found at the rural location with metabolic activation in winter (100.7 revertants/m^3) and summer (82.5 revertants/m^3). Two QF winter samples, taken at the industrial location, were toxic, since no bacterial growth was observed.

The mutagenic activity of the QF samples can also be expressed as activity per microgram PM10 collected on the filters (Table 3). Similar trends as for the activity per metrecube were obtained. In the winter

Table 3
Results of the *Salmonella* test: mutagenicity (response ± 95% confidence limits) of QF and PUF samples with (+S9) and without (−S9) metabolic activation[a]

Location	Season[b]	Mutagenic activity[c]					
		QF (revertants/m^3)		QF (revertants/μg PM10)		PUF (revertants/m^3)	
		−S9	+S9	−S9	+S9	−S9	+S9
Urban	W 1	44.4 ± 10.5	52.6 ± 3.5	1.75 ± 0.41	2.07 ± 0.14	–	4.3 ± 0.9
	W 2	36 ± 3.4	50.8 ± 3.2	0.79 ± 0.07	1.12 ± 0.07	–	23.3 ± 1.7
	W 3	38.2 ± 2.8	49 ± 9.1	1.16 ± 0.08	1.43 ± 0.28	–	7.7 ± 0.9
	S 1	35.3 ± 2.3	17 ± 2.9	0.85 ± 0.05	0.41 ± 0.07	8.6 ± 1.0	19.1 ± 2.4
	S 2	23.8 ± 1.6	14.7 ± 1.3	0.66 ± 0.04	0.41 ± 0.04	13.6 ± 0.9	17.5 ± 2.5
	S 3	21.3 ± 3.1	25.3 ± 2.2	0.68 ± 0.1	0.80 ± 0.07	16.3 ± 1.1	12 ± 1.6
Industrial	W 1	x	7.6 ± 1.7	x	0.17 ± 0.04	–	4.7 ± 0.5
	W 2	36.6 ± 2.8	54.1 ± 5.5	1.07 ± 0.08	1.58 ± 0.16	–	5.5 ± 0.9
	W 3	x	25 ± 3.1	x	0.92 ± 0.11	–	3.1 ± 0.3
	S 1	13.7 ± 0.7	8.9 ± 1.3	0.36 ± 0.02	0.23 ± 0.03	7.4 ± 0.5	12.4 ± 2.1
	S 2	15.1 ± 0.8	14.2 ± 1.6	0.43 ± 0.02	0.41 ± 0.04	16.6 ± 1.5	32.3 ± 1.8
	S 3	–	4.7 ± 0.8	–	0.39 ± 0.07	–	4.9 ± 0.5
Rural	W 1	42.3 ± 2.9	55.2 ± 6.2	1.4 ± 0.1	1.82 ± 0.2	–	3.7 ± 0.3
	W 2	58.2 ± 4.2	96.6 ± 11.3	1.02 ± 0.07	1.7 ± 0.2	–	4.1 ± 0.5
	W 3	–	10.9 ± 0.7	nd	nd	–	3.5 ± 0.5
	S 1	22.7 ± 2	21.2 ± 1.3	0.46 ± 0.04	0.43 ± 0.3	19.8 ± 2.7	60.3 ± 7
	S 2	6.6 ± 0.5	9.1 ± 0.9	0.34 ± 0.03	0.47 ± 0.05	–	3.7 ± 0.6
	S 3	5.8 ± 0.9	5.2 ± 0.8	0.37 ± 0.06	0.33 ± 0.05	–	6.5 ± 0.7

[a] Sampler type—QF: quartz filter; PUF: polyurethane foam.
[b] Each location was sampled three times in winter (W) and summer (S).
[c] (–) no mutagenic response; (x) toxic, no bacterial growth; (nd) not determined.

indirect activity was higher than direct activity ($P = 0.028$), whereas there was no significant difference in the summer. Activity in winter was higher than in summer ($P = 0.019$).

3.4. Genotoxic activity assessed by the Vitotox® assay

In the Vitotox® assay, the samples were tested at doses of 0.13, 0.25, 0.50 and 1% of the organic extracts or, respectively, 0.032, 0.065, 0.128 and 0.250 m^3 of air sampled. The evaluation of genotoxicity (lowest observed effect concentration, LOEC) and toxicity (effect concentration, EC50) is given in Table 4. Based on the calculations, as explained in Section 2, the genotoxic effects are presented as the lowest test dose giving a genotoxic result (LOEC) and the response (signal-to-noise ratio of the Rec and pr1 strain) per metrecube air. The raw data are available on the site http://allserv.ugent.be/~vdufour.

All PUF samples without metabolic activation were toxic. Although addition of S9 resulted in detoxification of these samples, only one sample (urban, winter 2) gave a genotoxic response. In contrast to the PUF extracts, the QF extracts gave genotoxic responses and less toxicity was observed, i.e. only three samples showed toxicity. The direct activity in winter was significantly higher ($P = 0.0006$). Metabolic activation (+S9) significantly reduced the genotoxic responses in QF samples, i.e. only the three samples with the highest direct response were still genotoxic after addition of S9.

Responses differed between winter and summer for the three locations. In the winter, the highest activity was found at the rural site and lower at the urban and industrial sites. During the summer, the highest activity was found at the urban site and almost no activity at the two other sites.

3.5. Modelling of mutagenicity and genotoxicity

Correlation analysis using PLS modelling was used to relate the mutagenic and genotoxic activity with

Table 4
Results of the Vitotox® assay: genotoxicity of QF and PUF samples with (+S9) and without (−S9) metabolic activation[a]

Location	Season[b]	QF[c]					PUF[c]				
		LOEC (%)		(rec/pr1)/m^3		EC50 (%)	LOEC (%)		(rec/pr1)/m^3		EC50 (%)
		−S9	+S9	−S9	+S9		−S9	+S9	−S9	+S9	
Urban	W 1	0.25	1	28.39	4.60	0.3	x	–	x	–	0.1
	W 2	0.5	+	22.12	6.10	0.12	x	1	x	7.4	0.13
	W 3	0.5	–	21.62	–	0.18	x	–	x	–	0.07
	S 1	0.5	–	19.65	–	0.21	x	–	x	–	0.21
	S 2	+	–	7.53	–	0.37	x	–	x	–	0.11
	S 3	1	–	9.00	–	0.28	x	–	x	–	0.31
Industrial	W 1	x	–	x	–	0.07	x	–	x	–	0.10
	W 2	0.25	1	29.35	6.80	0.20	x	–	x	–	0.12
	W 3	x	–	x	–	0.07	x	–	x	–	0.12
	S 1	+	–	11.19	–	0.24	x	–	x	–	0.09
	S 2	–	–	–	–	0.21	x	–	x	–	0.26
	S 3	x	–	x	–	0.16	x	–	x	–	nd
Rural	W 1	+	–	9.37	–	0.35	x	–	x	–	0.24
	W 2	0.13	0.50	69.60	13.30	0.17	x	–	x	–	0.19
	W 3	0.25	–	31.05	–	0.40	x	–	x	–	0.40
	S 1	0.10	–	7.52	–	0.78	x	–	x	–	0.45
	S 2	–	–	–	–	0.91	x	–	x	–	0.47
	S 3	–	–	–	–	0.72	x	–	x	–	0.64

[a] LOEC: lowest observed effect concentration; EC50: concentration where cell activity has dropped to 50% ($S/R = 0.5$); (+) probably genotoxic (Rec strain >1 while the Pr strain decreases); (–) no genotoxic effect; (nd) not determined.
[b] Each location was sampled three times in winter (W) and summer (S).
[c] Sampler type—QF: quartz filter; PUF: polyurethane foam.

the chemical composition of the samples (Table 5). Models based on the PAH concentrations and the *Salmonella* mutagenicity test of the filter samples predicted the indirect mutagenicity ($Q^2 = 0.702$) better than the direct mutagenicity ($Q^2 = 0.488$). Relating the indirect mutagenicity with the 10 mutagenic PAHs improved the model ($Q^2 = 0.741$). The models correlating the mutagenicity and chemical analyses of the PUF samples predicted direct ($Q^2 = 0.826$) and indirect ($Q^2 = 0.544$) activity quite well. The correlations or predictions were not improved by relating the indirect mutagenicity with the 10 mutagenic PAHs only.

Table 5
Summary of the PLS models developed, relating PAH concentrations and mutagenic/genotoxic responses

Result variable *Y*	No. of *X* variables	R^2	Q^2
Ames, +S9, QF	16	0.757	0.702
Ames, +S9, QF	10	0.754	0.741
Ames, −S9, QF	16	0.527	0.488
Ames, +S9, PUF	16	0.894	0.544
Ames, +S9, PUF	10	0.613	0.407
Ames, −S9, PUF	16	0.896	0.854
Vito, −S9, QF	16	0.898	0.84
Ames, +S9, QF + PUF	16	0.84	0.74
Ames, +S9, QF + PUF	10	0.499	0.459
Ames, −S9, QF + PUF	16	0.724	0.454

Y: mutagenic/genotoxic responses; *X*: PAH concentrations; Ames: *Salmonella* reverse mutation assay; Vito: Vitotox® test; R^2: correlation; Q^2: variance.

For the total (QF + PUF) indirect mutagenic activity of 18 samples and the corresponding PAH (16) concentrations, a good model was obtained ($Q^2 = 0.74$). The observed versus predicted total mutagenic response (revertants per metrecube) obtained by this model is shown in Fig. 2. Using only 10 PAH, the correlation and the prediction capability was significantly reduced. A similar model for the total direct activity was less powerful ($Q^2 = 0.454$).

A good model ($Q^2 = 0.898$) was developed for effects measured in the Vitotox® test based on the direct-acting activity of QF samples. Other models

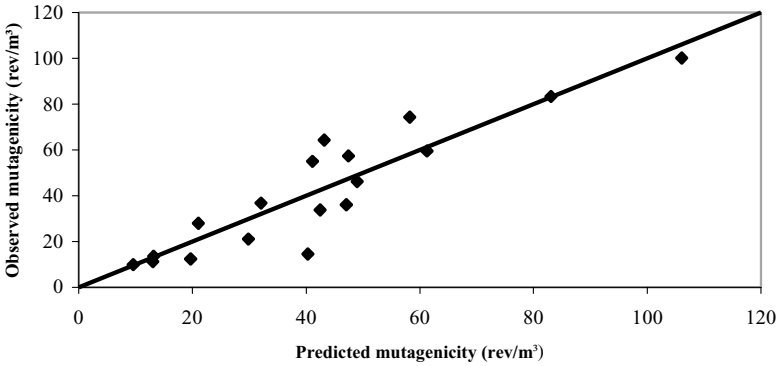

Fig. 2. The predictive capacity of the PLS model indicating observed vs. the predicted total (sum activity measured in QF and PUF sample) indirect mutagenicity.

based on the indirect-acting genotoxicity data were not relevant because almost no effects were observed with activation (+S9), or the samples showed severe toxic effects.

4. Discussion

This study reports genotoxic and mutagenic activity of ambient air in Flanders in combination with chemical analyses. The study was performed to evaluate the need to include biological testing in the Flemish environmental survey network and to assess health effects of environmental pollutants in an integrated way.

The complex mixtures of organic compounds to which we are exposed through air pollution are difficult to identify and to quantify. Therefore, chemical analyses in this study were limited to 16 PAHs, most of which are biologically active [20–23] and some even carcinogenic [6]. The seasonal and spatial patterns of these 16 PAHs were investigated. In the winter, the individual PAH concentrations were higher (except for ACE) than in the summer. Similar seasonal trends were also observed in previous studies [24–26]. Higher PAH concentrations in the winter are due to the contribution of domestic heating and to the specific meteorological conditions, i.e. sunlight intensities, temperatures and ozone concentration. These parameters under summer conditions increase volatilisation, the rates of chemical degradation and atmospheric dispersion [26], resulting in an artificial decrease of the measured concentrations at higher ambient temperatures. The total ($\sum$16) PAH concentrations were in the range 43.1–268.7 ng/m^3 in the winter and 20.9–88.9 ng/m^3 in the summer. These values correspond to values reported for polluted urban areas throughout the world [27–29].

Also the PAH profile was in agreement with those of other studies [26,28,30] i.e. lower molecular weight PAHs were the most abundant. The highest concentrations were measured for phenanthrene with values up to 66.8 ng/m^3, comparable with those previously reported (60 ng/m^3) by Müller et al. [28]. The concentrations of the heavier PAHs (five to six rings) were 10–100 times lower, however these are of higher concern because of their mutagenic and genotoxic activity.

In many studies B[a]P has been used as indicator compound for PAH exposure because of its strong carcinogenicity and close correlation with other PAHs [24,31]. With average concentrations of 1.4 ng/m^3 (0.1–5.3) in the winter and 0.1 ng/m^3 (0.02–0.1) in the summer, B[a]P levels in our study were among the lowest values reported in Europe. In Naples for instance, average concentrations were 2.67 ng/m^3 (0.09–12.18) during the winter and 1.09 ng/m^3 (0.03–4.02) in the summer [24], and concentrations comparable with those were found in Prague (Czech Republic) [32], Rome (Italy) [24], Thessaloniki (Greece) [27] and Teplice (Czech Republic) [32].

The total mutagenicity (QF + PUF) was higher in winter than in summer samples but the difference between both seasons was not as pronounced as in other studies. For example, Cerna et al. [33] reported mutagenic activities between 2 and 107 revertants/m^3 in the winter and between 2 and 23 revertants/m^3 for samples

taken in the summer. Comparable values were reported by Nardini and Clonfero [34]; 19–82 revertants/m^3 in winter and between 3 and 39 revertants/m^3 in summer samplings. Others like Binkova et al. [35] and Zhao et al. [36] described lower activities but still with a considerable difference between winter and summer, activities ranged between 13 and 25 revertants/m^3 in winter and 4–8 revertants/m^3 in summer and between 7 and 24 revertants/m^3 in winter and 2–8 revertants/m^3 in summer, respectively. In our study activities in the winter were at similar levels (4–100 revertants/m^3), but the activities measured during summer were two to four times higher than those reported elsewhere. This might be explained by the use of PUF cartridges next to the QF filters in our sampling campaign. Most papers reporting mutagenic activity of ambient air only used filters [33–40]. For the summer samples, 43% of the total activity was found in PUF extracts whereas only 10% was found on PUF cartridges in winter samples. This was confirmed by PAH concentrations in the different samples: in winter samples the higher molecular weight PAHs were almost exclusively found in the filter extracts. Conversely, in summer PUF extracts contained substantial amounts of these higher molecular weight PAHs, indicating that the higher total activities during summer are probably caused by these mutagens.

Indirect mutagenicity is usually attributed to PAHs [20], since these compounds need metabolic activation in order to show an effect. Next to the indirect-acting compounds other chemicals like nitro-PAHs and oxy-PAHs have direct mutagenic effect and do not need any metabolic activation [38]. In the air samples both types of mutagen were present: most extracts (mainly QF) showed direct and indirect mutagenicity.

Seasonal differences in mutagenic activity showed that the chemical composition or at least the ratio between the mutagens with direct or indirect activities are not identical in winter and summer. For the samples taken in the winter, the indirect mutagenicity was significantly higher than the direct activity, suggesting a considerable contribution of the PAHs. Indeed, the total level of the 10 carcinogenic PAHs as well as that of the individual PAHs, were two to seven times higher in winter than in summer. As discussed above, substantial activities were also measured in the summer and the mutagenicity levels were on average 60% of the levels observed during the winter. The mutagenicity level in the summer did not increase with metabolic activation, indicating that during this season the direct-acting mutagens contribute more to the total activity. It can be assumed that the levels of the nitro-derivatives are higher in the summer due to increased atmospheric reactions of PAHs with nitrogen oxides [33,39].

Fl, P, B[a]A, CH, B[b]F, B[k]F, B[a]P, DB[a,h]A, B[g,h,i]P and I[c,d]P all showed specific activity (revertants/μg) in the *Salmonella* test [20–23]. The sum of the concentrations of these 10 indirect-acting PAHs correlated quite well with the indirect (+S9) total mutagenic activity ($r = 0.73$, $P = 0.0004$). As discussed above, the levels of benzo(a)pyrene measured in our study were rather low. However, the mutagenic activity in Flanders was higher than expected based on the benzo(a)pyrene concentrations. The mutagenic activity (+S9) of air samples taken in a busy street in Copenhagen was on average 13.4 revertants/ng B[a]P for filter samples [40]. The values in our study were much higher with an average of 62.7 revertants ± 38.8/ng B[a]P. In the summer, the activity per nanogram B[a]P was much higher, i.e. 899 revertants ± 932/ng B[a]P, but this value is less precise due to the low B[a]P concentrations.

The air samples from the industrial and urban sites were expected to contain mutagenic compounds with higher activity and/or in higher quantities than those from the rural sites. However, the highest activity, both for QF and PUF samples, and the highest PAH concentrations were found in samples taken at the rural location, selected as a background site. This indicates that pollution is widespread in Flanders and that in developed Western nations exposure to pollution may be quite homogeneous. The presence of a military airport located a 4 miles from the sample site might have contributed to higher than expected PAH concentrations and mutagenic activity. This assumption is corroborated by the observation that the highest activity was found in samples taken when there was an east–northeast wind, which is the direction from the airport to the sampler. A similar situation was described by Zhao et al. [36], where high activity measured at a control site was explained by the presence of an international airport.

Two QF samples taken at the industrial location showed severe toxic effects in both the *Salmonella* assay and the Vitotox® test. Addition of S9 reduced the toxic effect but the resulting mutagenic response

was masked by lower bacterial growth. This toxic effect cannot be explained by the chemical analyses as no increased PAHs concentration was measured in these two samples. Probably other compounds, such as heavy metals, originating from a metallurgic industry nearby, caused this high toxicity.

There was no significant correlation between the results (QF samples) of the Vitotox® assay and the *Salmonella* test, although samples exhibiting an indirect genotoxic activity also showed a high mutagenic response (>50 revertants/m^3). The strong decline of the observed genotoxic activity in the presence of S9 suggests that the Vitotox® assay is mainly sensitive for direct-acting compounds, of which the reductive activation pathway can be disturbed by the oxidation enzymes in S9.

The Vitotox® results of the PUF samples without metabolic activation showed toxic effects for all samples, masking any possible genotoxic response. Toxicity was evaluated by the use of the bacterial strain TA104 pr1 [15] which provides a background light emission. All PUF samples (−S9) showed a reduction of activity, resulting in a strong decline of this background emission with increasing test dose. For the highest test doses (0.5 and 1%) with most samples the activity was too low to detect any genotoxic effect. At the lowest dose (0.13%) genotoxicy was detectable as little or no toxicity was found, however no genotoxic signals were observed, making further dilutions of no use.

With the PLS analyses we investigated possible correlations between the concentrations of 16 PAHs and the measured activities. The resulting models indicate which PAHs have an important contribution to the observed mutagenicity and genotoxicity. The model based on the direct mutagenicity of the filter samples showed a similar contribution for all 16 PAHs. The significant correlations with and predictions of direct activity based on the concentrations of indirect-acting PAHs indicates that these PAH concentrations are correlated with direct-acting mutagens like oxy- and nitro-PAHs. Contrary to this, the model using the indirect mutagenicity data showed that FL, P, B[a]A, CH, B[b]F, B[k]F, B[a]P, DB[a,h]A, B[g,h,i]P and I[c,d]P were the most important variables. These 10 PAHs are known indirect mutagens, only showing their effects in the presence of exogenous metabolic activation enzymes. The second PLS model, based on these 10 PAH, was developed and gave better correlations and predictions with the indirect mutagenicity results.

In conclusion, our work suggests that the *Salmonella* test is able to measure mutagenic activity of QF and PUF extracts and is suitable to use in a monitoring network. For both sample types, reproducible direct and indirect effects were found. With the Vitotox® assay, severe problems with toxicity of the PUF extracts were encountered and reduced genotoxic activity could only be detected in the presence of metabolic activation.

Compared to data from other regions, the mutagenicity levels in Flanders were high, especially in the summer. That our measurements during the summer resulted in values higher than those reported in most other studies can be explained by the use of PUF cartridges next to QF filters. In the summer, substantial amounts of higher molecular weight PAHs were found on the PUF cartridges, indicating that they were not sufficiently retained on the QF filters. As such, the use of a high-volume sampler for evaluating the mutagenic activity of ambient air in combination with only QF filters underestimates the mutagenic effects, especially during summer conditions.

The PAHs analysed in this study do not have any direct activity and the 10 known mutagenic PAHs were only responsible for a small part (1–2%) of the observed indirect activity: the B[a]P concentrations for example were very low compared with the measured activities. Using PLS analysis it was possible to make predictions of the observed activities based on the chemical analyses. Further improvement of these models requires more samples with different composition [18]. Chemical analyses provide essential information, but in order to come to an integrated assessment of the health effects of environmental pollution, bioassays need to be included. In an environmental monitoring network chemical analyses are restricted (practical and technical) to a limited number of compounds, only indicating the level of pollution. Bioassays allow us to assess the potential hazard of these complex environmental samples.

In this first study to measure the mutagenic/genotoxic activity of ambient air in Flanders, three different types of sites were selected. Results showed significant activities at all sites, with unexpectedly high values at the rural location, indicating that human exposure outside urban agglomerations is still significant.

Acknowledgements

This study was supported by the Science and Innovation Administration of the Flemish Community and the Flemish Environmental Agency. Special thanks go to Roland De Lathauwer for coordinating the sampling campaign, to Dr. Gilbert Desmet for the sample preparation and the chemical analyses and to Rinne De Bont for help with the literature study.

References

[1] M.S. Goldberg, R.T. Burnett, J.C. Bailar, J. Brook, Y. Bonvalot, R. Tamblyn, R. Singh, M.F. Valois, R. Vincent, The association between daily mortality and ambient air particle pollution in Montreal, Quebec, Environ. Res. 86 (2001) 26–36.

[2] A.J. Cohen, C.A. Pope, Lung cancer and air pollution, Environ. Health Perspect. 103 (Suppl.) (1995) 219–224.

[3] F.E. Speizer, Assessment of epidemiological data relating lung cancer to air pollution, Environ. Health Perspect. 47 (1983) 33–42.

[4] D. Zmirou, P. Masclet, C. Boudet, F. Dor, J. Dechenaux, Personal exposure to atmospheric polycyclic hydrocarbons in a general adult population and lung cancer risk assessment, J. Occup. Environ. Cancer Risk Assess. 4 (2000) 121–126.

[5] U. Vinitketkumnuen, K. Kalayanamitra, T. Chewonarin, R. Kamens, Particulate matter, PM10 & PM2.5 levels, and airborne mutagenicity in Chiang Mai, Thailand, Mutat. Res. 519 (2002) 121–131.

[6] IARC, Overall Evaluation of Carcinogenicity: An Updating of IARC Monograms 1 to 42, IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans, Suppl. 7, International Agency for Research on Cancer, Lyon, 1987.

[7] T. Nielsen, H.E. Jorgensen, M. Poulsen, F.P. Jensen, J.C. Larsen, M. Poulsen, A.B. Larsen, J. Schramm, J. Tonnesen, Traffic PAH and other mutagens in air in Denmark, Miljoproject no. 285, 1995, 140 pp.

[8] S. Monarca, D. Feretti, A. Zanardini, E. Falistocco, G. Nardi, Monitoring of mutagens in urban air samples, Mutat. Res. 426 (1999) 189–192.

[9] ISO/DIS 16362, Ambient air—determination of particle-phase polycyclic aromatic hydrocarbons by high-performance liquid chromatographic analysis, 2002.

[10] B.N. Ames, F.D. Lee, W.E. Durston, An improved bacterial test system for the detection and classification of mutagens and carcinogens, Proc. Natl. Acad. Sci. U.S.A. 70 (1973) 782–786.

[11] D.M. Maron, B.N. Ames, Revised methods for the *Salmonella* mutagenicity test, Mutat. Res. 113 (1983) 173–215.

[12] K. Mortelmans, E. Zeiger, The Ames *Salmonella*/microsome mutagenicity assay, Mutat. Res. 455 (2000) 29–60.

[13] P. Quillardet, M. Hofnung, The SOS chromotest: a review, Mutat. Res. 297 (1993) 235–279.

[14] D. Van der Lelie, L. Regniers, B. Borremans, A. Provoost, L. Verschaeve, The VITOTOX® test, a SOS-bioluminescence *Salmonella typhimurium* test to measure genotoxicity kinetics, Mutat. Res. 389 (1997) 279–290.

[15] L. Verschaeve, J. Van Gompel, L. Thilemans, L. Regniers, P. Vanparys, D. Van Der Lelie, VITOTOX® bacterial genotoxicity and toxicity test for the rapid screening of chemicals, Environ. Mol. Mutagen. 33 (1999) 240–248.

[16] Statsoft, Nonparametric statistics and distribution fitting, in: For Windows, vol. I, Tulsa, OK, USA 2000, pp. 1587–1638.

[17] Umetri AB, SIMCA-P for Windows, Graphical Software for Multivariate Process Modeling, Umeå, Sweden, 1996.

[18] I. Eide, G. Neverdal, B. Thorvaldsen, B. Grung, O.M. Kvalheim, Toxicological evaluation of complex mixtures by pattern recognition: correlating chemical fingerprints to mutagenicity, Environ. Health Perspect. 110 (2002) 985–988.

[19] M. Sjögren, L. Ehrenberg, U. Rannung, Relevance of different biological assays in assessing initiating and promoting properties of polycyclic aromatic hydrocarbons with respect to carcinogenic potency, Mutat. Res. 358 (1996) 97–112.

[20] H. Bartsch, C. Malaveille, A.M. Camus, G. Martel Planche, G. Brun, A. Hautefeuille, N. Sabadie, A. Barbin, Validation and comparative studies on 180 chemicals with *S. typhimurium* V79 Chinese hamster cells in the presence of various metabolising systems, Mutat. Res. 76 (1980) 1–50.

[21] M. Sakai, D. Yoshida, S. Mizusaki, Mutagenicity of polycyclic aromatic hydrocarbons and quinines on *Salmonella typhimurium* TA97, Mutat. Res. 156 (1985) 61–67.

[22] V.F. Simmon, In vitro mutagenicity assays of chemical carcinogens and related compounds with *Salmonella typhimurium*, J. Natl. Cancer Inst. 62 (1979) 893–899.

[23] M.F. Salamone, J.A. Heddle, M. Katz, The use of the *Salmonella*/microsomal assay to determine mutagenicity in paired chemical mixtures, Can. J. Genet. Cytol. 21 (1979) 101–107.

[24] E. Menichini, F. Monfredini, F. Merli, The temporal variability of the profile of carcinogenic polycyclic aromatic hydrocarbons in urban air: a study in a medium traffic area in Rome, 1993–1998, Atmos. Environ. 33 (1999) 3739–3750.

[25] M. Wada, H. Kido, N. Kishikawa, Assessment of air pollution in Nagasaki city, determination of polycyclic aromatic hydrocarbons and their nitrated derivatives, and some metals, Environ. Pollut. 115 (2001) 139–147.

[26] A.M. Caricchia, S. Chiavarini, M. Pezza, Polycyclic aromatic hydrocarbons in the urban atmospheric particulate matter in the city of Naples (Italy), Atmos. Environ. 33 (1999) 3731–3738.

[27] P. Georgiadis, S.A. Kyrtopoulos, Molecular epidemiological approaches to the study of the genotoxic effects of urban air pollution, Mutat. Res. 428 (1999) 91–98.

[28] J.F. Müller, D.W. Hawker, D.W. Connell, Polycyclic aromatic hydrocarbons in the atmospheric environment of Brisbane, Australia, Chemosphere 37 (1998) 1369–1383.

[29] C.J. Halsall, P.J. Coleman, B.J. Davis, V. Burnett, K.S. Waterhouse, P. Hardin-Jones, K.C. Jones, Polycyclic aromatic hydrocarbons in UK air, Environ. Sci. Technol. 28 (1994) 2380–2386.

[30] E. Manoli, D. Voutsa, C. Samara, Chemical characterization and source identification/apportionment of fine and coarse particles in Thessaloniki, Greece, Atmos. Environ. 36 (2002) 949–961.
[31] F. Pott, Environmental contamination by PAH-air, in: G. Grimm (Ed.), Environmental Carcinogens: Polycyclic Aromatic Hydrocarbons, CRC Press, Boca Raton, FL, USA, 1983, pp. 84–101.
[32] M. Cerna, A. Pastorkva, V. Vrbikova, J. Smid, P. Rössner, Mutagenicity monitoring of airborne particulate matter (PM10) in Czech Republic, Mutat. Res. 444 (1999) 373–386.
[33] M. Cerna, D. Pochmanova, A. Pastorkva, I. Benes, J. Lenicek, J. Topinka, B. Binkova, Mutagenicity monitoring of airborne particulate matter (PM10) in Czech Republic. Part I. Bacterial mutagenic potencies of organic compounds adsorbed on PM10 particulates, Mutat. Res. 469 (2000) 71–82.
[34] B. Nardini, E. Clonfero, Mutagens in urban air particulate, Mutagenesis 7 (6) (1992) 421–425.
[35] B. Binkova, M. Cerna, A. Pastorkova, R. Jelinek, I. Benes, J. Novak, R.J. Sram, Biological activities of organic compounds absorbed onto ambient air particles: comparison between the cities of Teplice and Prague during the summer and winter season 2000–2001, Mutat. Res. 525 (2003) 43–49.
[36] X. Zhao, Z. Wang, G. Ghen, H. Zhu, S. Jiang, J. Yao, Genotoxic activity of extractable organic matter from urban air particles in Shanghai, China, Mutat. Res. 514 (2002) 177–192.
[37] A. Buschini, F. Cassoni, E. Anceschi, L. Pasini, P. Poli, C. Rossi, Urban airborne particulate: genotoxicity evaluation of different size fractions by mutagenesis test on microorganisms and commet assay, Chemosphere 44 (2001) 1723–1736.
[38] L.D. Claxton, S. Warren, R. Zweidinger, J. Creason, A comparative assessment of Boise, Idaho, ambient air fine particle samples using the plate and microsuspension *Salmonella* mutagenicity assays, Sci. Total Environ. 275 (2001) 95–108.
[39] A. Feilberg, R.M. Kamens, M.R. Strommen, T. Nielsen, Modelling the formation, decay, and partitioning of semi-volatile nitro-polycyclic aromatic hydrocarbons (nitronaphthalenes) in the atmosphere, Atmos. Environ. 33 (1999) 1231–1243.
[40] T. Nielsen, H.E. Jorgensen, L. Grundahl, A.B. Jensne, P.A. Nielsen, J. Tonnesen, Traffic PAH and other air pollutants in the center of a large city, Report Riso-R-787(EN), Riso National Laboratory, Roskilde, Denmark, February 1995.